Convenient quantification of methanol concentration detection utilizing an integrated microfluidic chip

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A rapid and simple technique is proposed for methanol concentration detection using a PMMA (Polymethyl-Methacrylate) microfluidic chip patterned using a commercially available CO_2 laser scriber. In the proposed device, methanol and methanol oxidase (MOX) are injected into a three-dimensional circular chamber and are mixed via a vortex stirring effect. The mixture is heated to prompt the formation of formaldehyde and is flowed into a rectangular chamber, to which fuchsin-sulphurous acid is then added. Finally, the microchip is transferred to a UV spectrophotometer for methanol detection purposes. The experimental results show that a correlation coefficient of $R^2 = 0.9940$ is obtained when plotting the optical density against the methanol concentration for samples and an accuracy as high as 93.1% are compared with the determined by the high quality gas chromatography with concentrations in the range of $2 \sim 100$ ppm. The methanol concentrations of four commercial red wines are successfully detected using the developed device. Overall, the results show that the proposed device provides a rapid and accurate means of detecting the methanol concentration for a variety of applications in the alcoholic beverage inspection and control field. © *2012 American Institute of Physics*. [http://dx.doi.org/10.1063/1.4746246]

INTRODUCTION

The maturation of microelectromechanical systems (MEMS) technologies in recent decades has led to the development of many microfluidic systems for use in the food,[1–7] drug discovery,[8–14] environmental monitoring,[15–19] and biomedicine fields.[20–23] Typically, these systems comprise several functional devices designed to carry out specific tasks such as sample pretreatment and injection, species mixing, polymerase chain reaction, and cell/particle separation and counting.[24–34] Compared to their large-scale counterparts, microfluidic devices have numerous advantages, including a reduced sample and reagent consumption, an enhanced efficiency, an improved sensitivity, a shorter processing time, a lower power consumption, a greater portability, and a lower fabrication and operating cost.

The literature contains numerous proposals for integrated microfluidic devices for chemical and biological analysis applications.[35–45] Lin *et al.*[46] presented an efficient microfluidic mixer in which a freeze-quenching technique was used to trap the meta-stable intermediates formed during rapid chemical or biochemical reactions. Kim *et al.*[47] proposed a micro-mixer for the

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spectroscopic detection of glucose-catalyst reactions. Fu and Lin[48] presented a novel DNA digestion system in which the DNA and restriction samples were mixed via fixed and periodic switching DC electric fields. Wang *et al.*[49] presented an integrated microfluidic system for performing targeted ribonucleic acid (RNA) extraction and a one-step reverse transcription loop-mediated isothermal amplification (RT-LAMP) process in order to detect viruses from the tissue samples.

According to the standards set by the Taiwan Department of Alcoholic Beverage Control, the methanol content in general alcohol should not exceed 1000 ppm, while that in wine should be no more than 3000 ppm. The methanol concentration within alcoholic beverages is generally measured via gas chromatography (GC) or potassium permanganate oxidation.[50,51] The GC detection method is quick and highly accurate but involves a high capital equipment cost and expensive operating materials. As a result, it is impractical for small businesses and general users. Furthermore, the potassium permanganate oxidation method yields unreliable results due to the dissimilar reactive effects of the oxidation process on methanol and distilled alcohol, respectively Accordingly, various researchers have proposed adding methanol oxidase (MOX) to the methanol-containing solution rather than non-specific potassium permanganate, and then adding basic fuchsin to the resulting formaldehyde product such that the methanol concentration can be measured via colorimetry.[51,52]

In conventional test tube methods for methanol detection, washing and drying the test equipment is a time consuming and laborious process. Furthermore, the quartz glass substrates conventionally used for sample testing in a spectrophotometer are expensive. Accordingly, the present study proposes a fast, cost-effective and reliable method for methanol detection by means of a simple PMMA (Polymethyl-Methacrylate) microfluidic chip patterned using a commercially available CO_2 laser system. In the proposed device, methanol and MOX are injected simultaneously into a three-dimensional (3D) circular chamber by two micro syringe pumps and are mixed via a vortex stirring effect. Following a temperature-assisted oxidation process, the formaldehyde product is flowed into a rectangular chamber, where it is mixed with fuchsin-sulphurous acid. Finally, the microfluidic chip is transferred to a UV spectrophotometer for methanol detection purposes. The experimental results indicate that the linear expression R^2 can approximate 0.9940 using the proposed integrated microfluidic chip and approximate 0.9950 using the traditional method when the mixture of two-unit methanol oxidase (MOX) and basic fuchsin (BF, Schiff method) for various concentrations of methanol detection. The validity of the proposed microfluidic approach is demonstrated by comparing the detection results obtained for samples with known methanol concentrations in the range of $2 \sim 100$ ppm with those obtained using the current proposed method.

FABRICATION AND EXPERIMENTAL DETAILS

Figure 1 presents a photograph of the proposed microfluidic chip. The microchannel configuration was designed using commercial AUTOCAD (2011) software and was then converted into a machining pattern via COREL GRAPHICS SUITE 11 software. The microchannels were scribed into PMMA substrates[53] using a VII-12 CO_2 laser system (Giant Technologies Incorporated), operated in a continuous mode with a maximum power output of 12 W and a wavelength of 10 500 nm. Prior to the ablation process, scribing trials were performed using a focused laser beam and a defocused laser beam, respectively. The results showed that the focused beam method yielded an average surface roughness of more than 2000 Å. By contrast, the defocused laser beam method achieved a surface roughness of less than 40 Å when using a defocused height of 40 mm. Accordingly, the integrated microfluidic chip was patterned using the defocused laser beam method. In performing the ablation process, the output power and laser travel speed were specified as 4 W and 120 mm/s, respectively, and the defocused length was set as 40 mm. The total machining time was less than 2 min. (Note that a detailed description of the defocused laser beam ablation method is presented elsewhere.[54])

As shown in Fig. 2, the microfluidic chip comprised three PMMA substrates, namely, one upper substrate (Substrate #1, thickness 1.6 mm), one middle substrate (Substrate #2, thickness

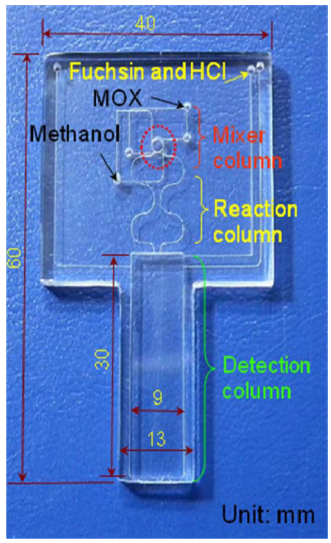

FIG. 1. Photograph of methanol detection microfluidic chip.

6 mm), and one lower substrate (Substrate #3, thickness 1.6 mm). The diameter of the three inlet ports for the methanol sample, MOX (from *Pichia pastoris,* Sigma, St. Louis, MO, USA) reagent, and fuchsin-sulphurous acid (fuchsin/HCl) solution, respectively, was specified as 1.5 mm in every case (see Substrate #1 in Fig. 2(a)). Meanwhile, the mixing chamber was designed with a diameter of 2 mm (see Substrate #2 in Fig. 2(b)). In addition, the rectangular collection tank used to store the MOX reagent/methanol mixture exiting the mixing chamber was designed with dimensions of 9 mm × 30 mm. Finally, a mixing chamber connecting the microchannels was ablated in Substrate #3 (see Fig. 2(c)). Following the ablation process, the three PMMA substrates were carefully aligned and sealed using a hot-press bonding technique (see Figs. 2(d) and 2(e)). In performing the bonding process, the three substrates were inserted between two thick glass plates and maintained under a pressure of 0.5 kg/cm^2 for 10 min as the temperature was progressively increased to 101 °C. The pressure was then increased to 7.5 kg/cm^2 for 15 min with no further change in the temperature. Finally, the sealed microchip was trimmed using a focused laser beam (laser power 10 W; laser movement speed 80 mm/s) to

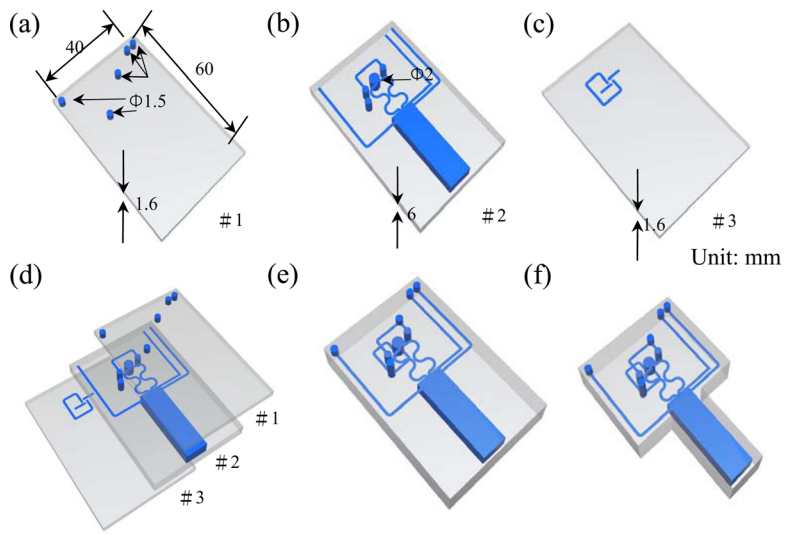

FIG. 2. Schematic overview of fabrication process used to realize the integrated microfluidic chip.

form a T-shaped microfluidic device (see Fig. 2(f)). As shown, the detection region of the T-shaped device was assigned dimensions of 13 mm × 27.5 mm (i.e., the same dimensions as the cuvette used in the macro-scale methanol detection experiments performed in the present study).

Figure 3 illustrates the major steps involved in fabricating the microfluidic chip and performing the methanol concentration detection experiments. The sample reagents comprised methanol, MOX, basic fuchsin, HCl, and deionized (DI) water. The following samples and reagents were prepared (Fig. 3(c)): (1) methanol with concentrations ranging from 2 to 100 ppm, prepared by mixing methanol and DI water in appropriate quantities; (2) prepared by mixing 300 μl of MOX (2 unit/ml in 0.01 M phosphate buffer, *p*H 7.5); and (3) basic fuchsin (in 1% Na_2SO_3 and 1% H_2SO_4 solution)/DI water, prepared by mixing basic fuchsin of DI water at a temperature of 45 °C, and then adding 1 N HCl solution. As shown in Figs. 3(d) and 3(e), the methanol detection chip was installed on an experimental platform comprising an aluminum base, a transparent PMMA top plate, four wing nuts, three NanoPort connectors (N-333, Upchurch Scientific, Oak Harbor, WA), and a micro-heater. The inlet ports on the microchip were connected to syringe pumps (KDS 100, USA) via appropriately sized Teflon tubing and were sealed using to prevent sample leakage. In performing the methanol detection experiments, methanol and MOX were injected into the microchip and mixed within the circular micro-chamber for 5 min. The mixture exiting the microchamber was maintained at a constant temperature of 45 °C for approximately 25 min in order to enhance the methanol/MOX oxidation reaction (i.e., $CH_3OH+O_2 \xrightarrow{MOH} HCOH+H_2O_2$.) The reaction products were flowed into the rectangular collection tank and mixed with fuchsin-sulphurous acid in order to form samples for colorimetric observations. The syringe pumps were then turned off. The fuchsin-sulphurous acid syringe pump was then turned off and the microfluidic chip removed from the experimental platform and inserted into the detection trough of a commercial UV spectrophotometer (Model U-2000, Tokyo, Japan) in order to observe the corresponding absorption spectrum (see Fig. 3(f)).

COMPUTATIONAL SIMULATIONS

Computational fluid dynamics (CFD) simulations were performed to examine the flow streamlines and concentration contour distributions within the mixing chamber in order to estimate the mixing performance at various Reynolds numbers. In performing the simulations, the flow field within the mixing chamber was obtained by solving the 3-D incompressible Navier-

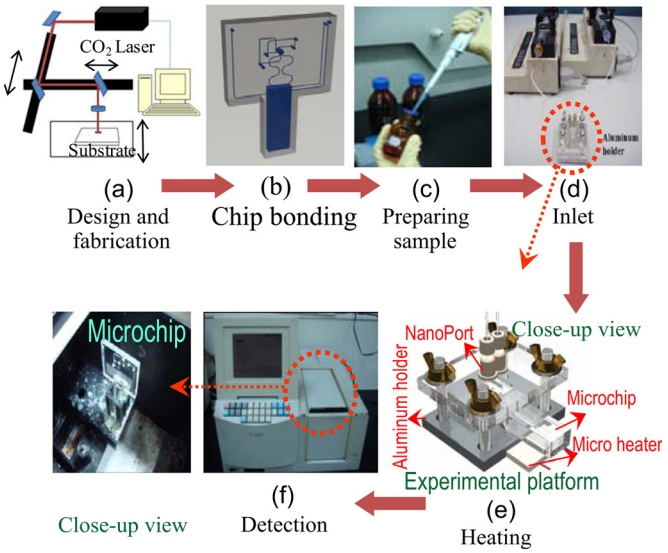

FIG. 3. Schematic illustration showing major steps in chip fabrication and methanol concentration detection procedures.

Stokes equations. (Note that a detailed description of the relevant governing equations and the numerical solution procedure is presented in previous studies by the current group.[25])

RESULTS AND DISCUSSIONS

The mixing effect within the circular micro-chamber was investigated experimentally by injecting 10^{-6} M Rhodamine B fluorescence dye and DI water into the chamber at a Reynolds number of Re = 4. The mixing process was observed using a fluorescent microscope (E-400, Nikon, Japan) fitted with an image acquisition card (DVD PKB, V-gear, Taiwan). Figures 4(a) and 4(b) present the numerical and experimental results for the species mixing effect within the micro-chamber after 2 s and 10 s, respectively. The results indicate that a complete mixing of the two species is obtained after 10 s. Moreover, it is observed that a good qualitative agreement exists between the numerical and experimental results in both cases. Thus, the basic validity of the numerical model is confirmed.

Figure 4(c) compares the numerical and experimental results obtained for the variation of the mixing ratio with the Reynolds number at a cross-section located 1 mm downstream from the outlet of the mixing chamber. Note that the normalized concentrations of the original (i.e., unmixed) Rhodamine B and DI water solutions are equal to 1 and 0, respectively. Thus, a mixing ratio of 0.5 indicates that the two species are fully mixed. In general, the mixing ratio of two species can be quantified as follows:[55]

$$\sigma = \left(1 - \frac{\int_A |C - C_\infty| dA}{\int_A |C_0 - C_\infty| dA}\right) \times 100\%, \tag{1}$$

where C is the species concentration profile across the width of the microchannel, and C_o and C_∞ are the species concentrations in the completely unmixed (0 or 1) and completely mixed (0.5) states, respectively. Figure 4(c) shows that the vortex structure results in an effective mixing of the two species at even low values of the Reynolds number. Moreover, it is observed that the mixing performance is enhanced as the Reynolds number is increased due to the corresponding increase in the intensity of the vortex structure. Overall, the results show that the optimal mixing ratio (~95%) is obtained at Reynolds numbers greater than or equal to 4. Thus, in all the remaining experiments and simulations, the Reynolds number was specified as Re = 4.

Figure 5 compares the steps involved in the traditional macro-scale methanol detection method (Fig. 5(a)) with those in the proposed microfluidic chip-based method (Fig. 5(b)). In the

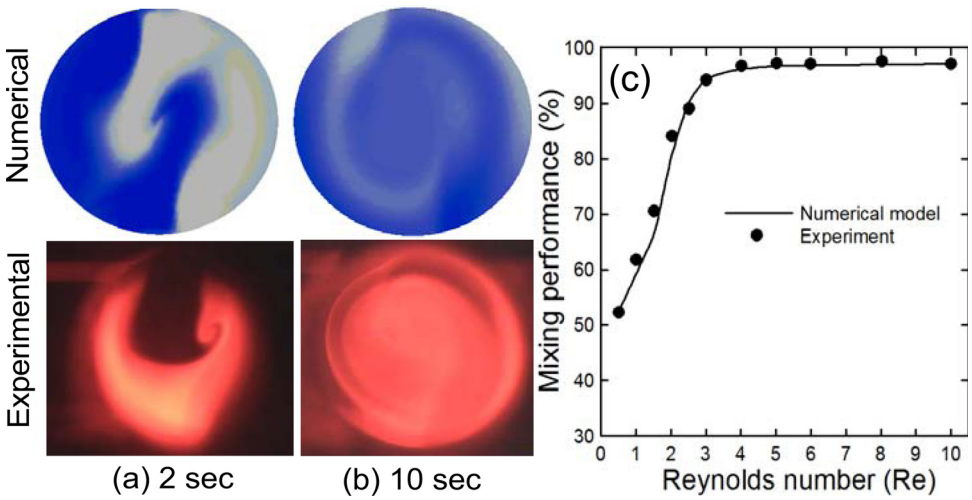

FIG. 4. Numerical and experimental results for mixing effect within micro-chamber after: (a) 2 s, (b) 10 s, and (c) The mixing efficiency within micro-chamber given Reynolds numbers in the range of Re = 0.5 ~ 10.

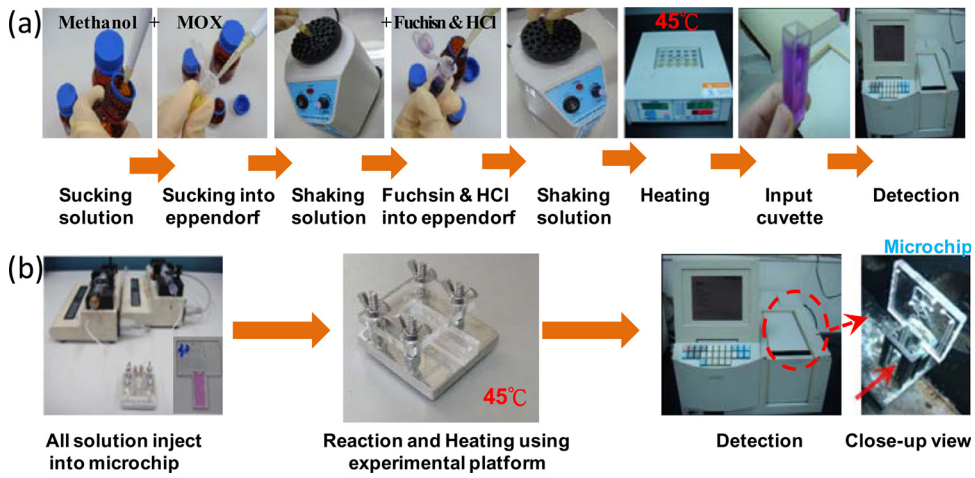

FIG. 5. Comparison of procedural steps in: (a) traditional methanol concentration detection method; and (b) microfluidic methanol concentration detection method.

traditional method performed in this study, 1 ml of methanol sample (with a concentration of $2 \sim 100$ ppm) was injected into an Eppendorf pipette with 1 ml of MOX reagent. The pipette was shaken mechanically for approximately 30 min under room temperature conditions ($\sim$25 °C) to ensure a thorough species mixing. Basic fuchsin (1 ml, 0.1%) and HCl (1 ml, 1N) (fuchsin-sulphurous acid) were then added to the pipette, which was then shaken for a further 1 min. The pipette was then heated at a constant temperature of 45 °C for approximately 120 min. Finally, the reacted solution was suctioned from the pipette into a cuvette and the cuvette was then inserted into a UV spectrophotometer (560 nm) to analyze the absorbance spectrum. The entire procedure required approximately 2.5 h for each sample.

In the microfluidic methanol detection method (Fig. 5(b)), 0.3 ml of methanol solution (with a concentration of $2 \sim 100$ ppm) and 0.3 ml MOX reagent were injected into the mixing chamber at a Reynolds number of $\mathrm{Re} = 4$. In order to acquire stable OD value in the detection processes, the mixture maintained at a constant temperature of 45 °C using the experimental platform for approximately 15 min. A basic fuchsin (0.3 ml, 0.1%)/HCl (0.3 ml, 1N) (fuchsin-sulphurous acid) solution was then injected into the rectangular collection tank, which was maintained at 45 °C using the experimental platform for a further 25 min to prompt a colorimetric reaction. Finally, the microfluidic chip was removed from the experimental platform and inserted into the detection trough of the spectrophotometer in order to measure the optical density (OD) value. Thus, the reaction process for the samples was completed within approximately 40 min.

Figure 6(a) presents the variation of the OD value with the methanol concentration for the samples prepared using the traditional macro-scale method and proposed microfluidic method from $2 \sim 100$ ppm. (Note that the OD results represent the average value of 5 different measurements for each sample.) Applying a regression analysis technique to the experimental data, it is found that the OD value (Y) and the methanol concentration (X) are related as follows $\mathrm{Y} = 0.0082\mathrm{X} + 0.2888$ for using traditional macro-scale method. Moreover, the correlation coefficient is found to be $\mathrm{R}^2 = 0.9950$. In addition, to the absorbance values obtained for the standard (i.e., control) methanol samples, Figure 6(a) also shows the detection results obtained using the proposed microfluidic method for methanol samples with concentrations ranging from 2 to 100 ppm. From inspection, the OD value and the methanol concentration are found to be related via the relationship $\mathrm{Y} = 0.0079\mathrm{X} + 0.2888$. Moreover, the correlation coefficient is found to have a value of $\mathrm{R}^2 = 0.9940$. It is noted that the correlation coefficient for the proposed microfluidic method is very close to that for the traditional macro-scale method, and the detection limit can be approached 1 ppm. In other words, the precision of the miniaturized microfluidic methanol concentration detection system is confirmed.

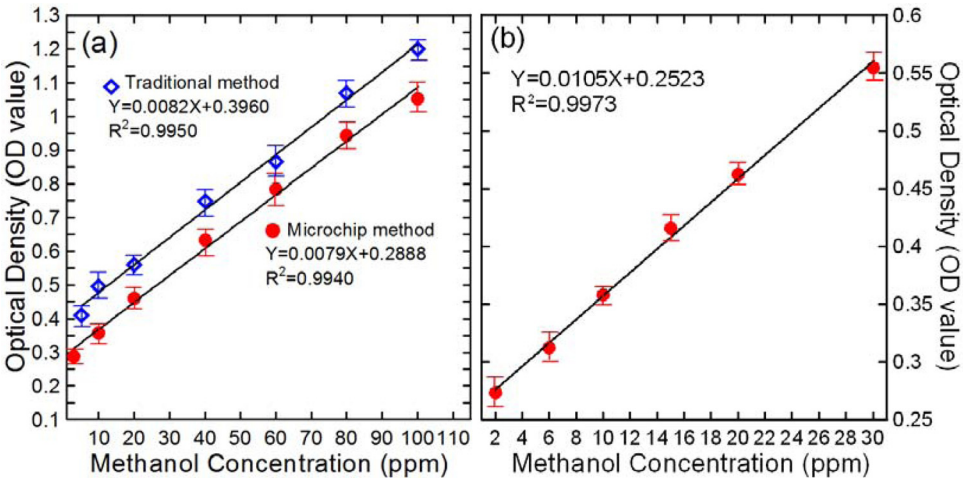

FIG. 6. (a) Variation of optical density with methanol concentration given standard conventional methanol concentration detection system and obtained using proposed microfluidic method. (b) Variation of optical density with methanol concentration with methanol concentrations in the range of 2-30 ppm. (Note that the results represent the average values obtained from five separate experiments for each sample.)

The suitability of the proposed microfluidic methanol concentration detection chip for real-world methanol detection applications was investigated using four commercial red wine samples, namely, sample #1 (Chaerrs, Feng Cheng Co., Taiwan), sample #2 (Rosticity, Hua Shan Quan Co., Taiwan), sample #3 (Blueberry, Chio Shuen Co., Taiwan), and sample #4 (Cherry, Chio Shuen Co., Taiwan). Accordingly, it is necessary to rework the calibration curve presented in Fig. 6(b) for an amended concentration range of $2 \sim 30$ ppm. From inspection, the absorbance value and methanol concentration are found to be related via the relationship $Y = 0.0105\,X + 0.2523$. (Note that this equation is referred to hereafter as the *experimental group equation*.) Moreover, the correlation coefficient is found to have a value of $R^2 = 0.9973$. For reference purposes, the methanol concentrations of the four samples were also evaluated using the by the Center for Agriculture and Aquaculture Product Inspection and Certification (CAAPIC, ISO/IEC 17025 Accreditation by Taiwan Accreditation Foundation) at National Pingtung University of Science and Technology in Taiwan using a GC method. The methanol concentration results obtained using the two different methods are presented in Table I (Note that in the proposed microfluidic case, the results correspond to the average value obtained from five separate measurements.) As shown, the absorbance values of sample #1, sample #2, sample #3, and sample #4 are 0.2943, 0.3446, 0.3120, and 0.3378, respectively. Substituting these two values into the *experimental group equation*, the corresponding methanol concentrations are found to be 4.000 ppm, 8.791 ppm, 5.682 ppm, and 8.147 ppm, respectively. According to the results obtained from CAAPIC, the two samples have methanol concentrations of 4.3 ppm, 9.1 ppm, 6.0 ppm, and 8.4 ppm, respectively. It was found that the accuracy of the two sets between

TABLE I. Methanol concentration results obtained for four commercial red wines using proposed microfluidic system and GC system, respectively. Note that for each sample, the absorbance results represent the average value of 5 different measurements.

Samples	Average OD value	Microfluidic system (ppm)	NPUST CAAPIC detection[a] (ppm)
Red wine #1	0.2943	4.000	4.3
Red wine #2	0.3446	8.791	9.1
Red wine #3	0.3120	5.682	6.0
Red wine #4	0.3378	8.147	8.4

[a]NPUST: National Pingtung University Science Technology, CAAPIC: Center for Agriculture and Aquaculture Product Inspection and Certification.

proposed microfluidic system and GC system were 93.1%, 96.6%, 94.7%, and 96.9% respectively. (Note that the accuracy (%) is obtained as *Accuracy* $(\%) = \left(1 - \left|\frac{microfluidic\ method - GC\ method}{GC\ method}\right|\right)$. In other words, the precision of the proposed microfludic system when applied to real-world samples is confirmed.

CONCLUSIONS

This study has presented a rapid and low-cost technique for methanol concentration detection using a PMMA microchip patterned using a commercial CO_2 laser system. An experimental platform has been created for multiple reaction process. In the proposed approach, methanol and MOX are mixed within a circular micro-chamber and heated to a temperature of 45 °C for 15 min in order to prompt a reaction between them. The resulting formaldehyde is then mixed with fuchsin-sulphurous acid and maintained at 45 °C for a further 25 min in order to induce a colorimetric reaction. Finally, the microchip is transferred to a UV spectrophotometer to observe the corresponding absorption spectrum. A series of methanol concentration detection tests have been performed using the proposed microfluidic approach for methanol samples with concentrations ranging from 2 to100 ppm. The experimental result has shown that the correlation coefficient obtained when plotting the optical density of the colorimetric samples against the methanol concentration has a value $R^2 = 0.9940$ when using the microfluidic detection method. Moreover, it has been shown that the methanol concentration measurements obtained by the proposed system for four commercial red wines deviate by no more than 6.9% from the measurements obtained using a commercial GC system. In addition, the dimensions of the microchip can be easily tailored to suit the differing trough dimensions of different commercial spectrophotometers by adjusting the machining pattern used by the laser scriber. Thus, overall, the method proposed in this study provides a simple, versatile, and accurate tool for evaluating the methanol concentration in various applications within the alcoholic beverage inspection and control field.

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